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Antibacterial effect of *Moringa oleifera* on *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from raw milk and some dairy products with special reference to biofilm gene expression

Rowyda Mohamed Yousry Elshazely[1*], Ibrahim H. Amer[2], Salah F. A. Abd-El Aal[2] and Asmaa B. M. B. Tahoun[2]

[1]*Dakahlia Veterinary Directorate, Mansoura City, Egypt*
[2]*Department of Food Hygiene, Safety and Technology, Faculty of Veterinary Medicine, Zagazig University, Zagazig City, Egypt*

ABSTRACT

Background: *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) are well defined as food poisoning pathogens that are highly resistant and need continuous studies.

Aim: The purpose of the work was to examine phenotypic and genotypic characteristics of both *P. aeruginosa* and *S. aureus*, and treatment trials with medicinal plants.

Methods: Samples were examined for isolation of *P. aeruginosa* and *S. aureus* on selective media followed by biochemical confirmation, biofilm formation, genes detection**,** and expression of *P. aeruginosa pslA* biofilm gene was performed by quantitative real-time polymerase chain reaction after treatment with 0.312 mg/ml *Moringa oleifera* aqueous extract as a minimum inhibitory concentration.

Results: The highest isolation rate of *P. aeruginosa* was 20% from both raw milk and Kariesh cheese, followed by 16% and 12% from ice cream and processed cheese, respectively, while the highest isolation rate of *S. aureus* was 36% from raw milk followed by 28% in ice cream and 16% in both Kariesh cheese and processed cheese. 30% of *P. aeruginosa* isolates were biofilm producers, while only 21% of *S. aureus* isolates were able to produce biofilm. The *P. aeruginosa* isolates harbor virulence-associated genes *nan1*, *exoS*, *toxA*, and *pslA* at 100%, 80%, 40%, and 40%, respectively. *Staphylococcus aureus SEs* genes were examined in *S. aureus* strains, where *SEA* and *SEB* genes were detected with 60%, but no isolate harbored *SEC*, *SED*, or *SEE*. The significant fold change of *P. aeruginosa pslA* expression was 0.40332 after treatment with *M. oleifera* aqueous extract.

Conclusion: *Pseudomonas aeruginosa* and *S. aureus* harbor dangerous virulence genes that cause food poisoning, but *M. oleifera* extract could minimize their action.

Keywords: Antibacterial, *Moringa oleifera*, Biofilm gene expression, *Staphylococcus aureus*, *Pseudomonas aeruginosa.*

Introduction

Food-borne pathogens can easily take their way to milk and dairy products and contaminate them (Painter *et al*., 2013). Micro-organisms express their virulence by the act of some genes that encode products inside the host body (Muthu *et al*., 2014). Recently diseases caused by food-borne pathogens have a great attention globally (Bhunia, 2018). For this, regular checking of milk and dairy products' microbial content and virulence genes became of high value and concern (Momtaz *et al*., 2012). *Staphylococcus aureus* has been proven to be the main cause of many food poisoning cases and outbreaks that take place when having contaminated meals that contain milk or dairy products (Vazquez-Sanchez *et al*., 2014), due to their high content of carbohydrates and protein (Fletcher *et al*., 2015). Gastro-enteric disturbances are induced after having meals containing staphylococcal heat-stable enterotoxins (SEs) (Mossong *et al*., 2015). *Staphylococcus aureus* can produce about 23 enterotoxins (Podkowik *et al*., 2013), where the most prominent ones are A (SEA) and D (SED), while B (SEB), C (SEC), and E (SEE) come after them (Balaban and Rasooly, 2000). A highly genetic plasticity *Pseudomonas aeruginosa* easily adapts to various environments, so it is easily isolated from soil and water and it has been found colonized in various living animals (Streeter and Katouli, 2016). Pathogenicity of *P. aeruginosa* is related to biofilm formation [where alginates enter the colonies composition to antagonist antibiotics medication and host immunity (Moradian

***Corresponding Author:** Rowyda Mohamed Yousry Elshazely. Dakahlia Veterinary Directorate, Mansoura City, Egypt. Email: *rowyda.vet2021@gmail.com*

et al., 2012)], numerous membranes existence, and extracellular virulence factors production (Khattab *et al*., 2015) mainly exotoxins which are extremely toxic causing tissue damage (Sato and Frank, 2004), acute infection and food poisoning (Nikbin *et al*., 2012). ExoS (exoenzyme S) is one of three ADP-ribosyltransferases (Wolska *et al*., 2012) that are produced by *P. aeruginosa* type III secretion system into the cytoplasmic matrix of epithelial cells directly (Fadhil *et al*., 2016). The role of *nan1* (neuraminidase) gene is starting colonization and facilitating the pathogen adherence easily to the respiratory tract and buccal epithelium, as it is encoded to sialidase causing long term infection (Moradian *et al*., 2012). Biofilm initiation and then keeping it is done by the *pslA* gene (polysaccharide synthesis locus A) (Yang *et al*., 2011). Multidrug-resistant (MDR) pathogens are in continuous progress in reducing the effect of antibiotics (Brown and Wright, 2016) increasing health problems and deaths (Ali *et al*., 2018). Recently, alternative medicinal plants have been of great concern instead of antibiotics (Zuo *et al*., 2008) like *Moringa oleifera* plant which is called the miracle tree due to its role in nutrition and medicine (Oyeyinka and Oyeyinka, 2018), besides its leaves aqueous extract has been confirmed to be consumed orally safely in high doses (Awodele *et al*., 2012). It has a proven bactericidal effect and several medicinal uses (Suarez *et al*., 2005). Several health benefits that gained after having *Moringa* leaves besides its great nutritional value (Teixeira *et al*., 2014), which cannot be counted as it acts as a diuretic, anti-neoplastic, anti-diabetic, anti-inflammatory, antimicrobial, antioxidant, antihypertensive, anti-hyperlipidemia, antipyretic, antiulcer, cardio protectant, and hepato-protectant (Vongsak *et al*., 2014). In our study, we have examined the prevalence of food poisoning pathogens (*S. aureus* and *P. aeruginosa*), their biofilm reaction, and virulence genes in milk and some dairy products, then, we have examined *M. oleifera* leaves aqueous extract effect on the recovered isolated *P. aeruginosa*.

Materials and Methods

Sample collection

A 100 samples of raw milk, Kariesh cheese, processed cheese, and ice cream (25 each), were randomly purchased from small outlets, vendors, and markets in Sharkia and Dakahlia Provinces, Egypt, which were examined during the period from November 2021 to April 2022.

Bacteriological phenotypic examination

Bacteriological examination

One milliliter of the examined sample was enriched in 9 ml of trypton soya (TS) broth, then a loopful was streaked onto both HiCrome™ Staph Selective Agar (Himedia, Mumbai) (M1913) (APHA, 2015) and Pseudomonas Agar Base (Himedia, Mumbai) (M085) with glycerol and CetriNix Supplement (Himedia, Mumbai) (FD029) (APHA, 2015). The suspected colonies were subcultured on trypticase soya broth and incubated at 30°C for 24 hours to obtain subculture. The suspected isolates were then biochemically examined via cystine-lactose-electrolyte-deficient (C.L.E.D) agar (Thomson and Miller, 2003).

Congo red agar (CRA) test for biofilm production

Cultures were streaked onto modified CRA medium plates (Mariana *et al*., 2009).

Molecular examination of suspected isolates

Molecular identification of isolated pathogens

Isolates which were biofilm producers (five isolates of each pathogen, for *S. aureus* two isolates were taken from raw milk samples, two from Kariesh cheese samples, and one from ice cream sample, while *P. aeruginosa* isolates were three from raw milk samples, one from processed cheese sample, and one from ice cream sample) were further examined in the Polymerase Chain Reaction (PCR) Unit, Animal Health Research Institute, Zagazig, Sharkia, Egypt. Extraction of bacterial DNA was done according to manufacturer's guidelines. 16S rRNA for both *S. aureus* (Monday and Bohach, 1999) and *P. aeruginosa* isolates (Ghadaksaz *et al*., 2015).

Table 1. Oligonucleotide primers, their specific sequence, and the specific amplified product of *P. aeruginosa*.

Pseudomonas genes	Primer sequence 5'–3'	Amplified product	Reference
16sRNA	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	925 bp	Ghadaksaz *et al*. (2015)
exoS	CTT GAA GGG ACT CGA CAA GG ' TTC AGG TCC GCG TAG TGA AT	504 bp	
nan1	AGG ATG AAT ACT TAT TTT GAT TCA CTA AAT CCA TCT CTG ACC CGA TA	1,316 bp	Khattab *et al*. (2015)
toxA	GGT AAC CAG CTC AGC CAC AT TGA TGT CCA GGT CAT GCT TC	352 bp	
PslA	TCCCTACCTCAGCAGCAAGC TGTTGTAGCCGTAGCGTTTCTG	656 bp	Ghadaksaz *et al*. (2015)

Table 2. Oligonucleotide primers, their specific sequence, and the specific amplified product of *S. aureus*.

Staphylococcus genes	Primer	Primer sequence (5'–3')	Length of amplified product	Reference
16S rRNA	F	GTA GGT GGC AAG CGT TAT CC	228 bp	Monday and Bohach (1999)
	R	CGCACATCAGCGTCAG		
Sea	SEA-F	GGTTATCAATGTGCGGGTGG	102 bp	Mehrotra *et al*. (2000)
	SEA-R	CGGCACTTTTTTCTCTTCGG		
Seb	SEB-F	GTATGGTGGTGTAACTGAGC	164 bp	
	SEB-R	CCAAATAGTGACGAGTTAGG		
Sec	SEC-F	AGATGAAGTAGTTGATGTGTATGG	451 bp	
	SEC-R	CACACTTTTAGAATCAACCG		
Sed	SED-F	CCAATAATAGGAGAAAATAAAAG	278 bp	
	SED-R	ATTGGTATTTTTTTTCGTTC		
See	SEE-F	AGGTTTTTTCACAGGTCATCC	209 bp	
	SEE-R	CTTTTTTTTCTTCGGTCAATC		

Table 3. Cycling conditions of the different primers during PCR of *P. aeruginosa*.

No.	*Pseudomonas* genes	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
1	16s rRNA	95°C 10 minutes	94°C 1 minute	52°C 1 minute	72°C 1 minute	30	72°C 10 minutes
2	exoS	94°C 5 minutes	94°C 30 seconds	55°C 30 seconds	72°C 30 seconds	35	72°C 7 minutes
3	nan1	95°C 10 minutes	94°C 1 minute	58°C 1 minute	72°C 1 minute	30	72°C 10 minutes
4	toxA	95°C 10 minutes	94°C 1 minute	58°C 30 seconds	72°C 1 minute	35	72°C 10 minutes
5	pslA	94°C 5 minutes	94°C 30 seconds	60°C 40 seconds	72°C 30 seconds	35	72°C 7 minutes

Virulence genes PCR amplification

SEs A to *E* virulence-associated genes were examined in *S. aureus* isolates (Mehrotra *et al*., 2000). *Pseudomonas aeruginosa* isolates were examined for *toxA* (exoenzyme A), *exoS*, *nan1* virulence-associated genes (Khattab *et al*., 2015) and *pslA* biofilm gene (Ghadaksaz *et al*., 2015) (Tables 1–4).

Moringa oleifera treatment

It was done according to Shah *et al*. (2015).

Extract preparation (0.5%)

Preparation of P. aeruginosa isolate

Pseudomonas aeruginosa isolate used in this experiment is highly virulent and was isolated from raw milk during this study, confirmed biochemically and by the conventional method of PCR, in addition, it harbored *exoS* and *nan1* virulence genes, moreover it reacted as a medium biofilm producer on CRA and was positive for *pslA* biofilm gene. The isolate was incubated in TS broth at 35°C overnight.

Preparation of S. aureus isolate

Staphylococcus aureus isolate examined is highly virulent, where it was isolated from a Kareish cheese sample, and biochemically confirmed, also, it was positive for 16S rRNA and SEB gene and it was a strong biofilm producer. That isolate was incubated in a TS broth tube and at 35°C overnight.

Determination of antibacterial activity of aqueous extract of M. oleifera

Minimum inhibitory concentration (MIC) and sub inhibitory concentration (SIC) by micro-dilution method

Serial dilution of 100 µl of 0.5% *M. oleifera* aqueous extract was performed in 96 well plates (TechnoPlastic Products, Switzerland), then it was inoculated with 5 × 10^5 CFU/ml of *P. aeruginosa* and *S. aureus* apart and overnight incubation was performed at 37°C, inoculation of each well were performed on each specific media to determine MIC and SIC; the lowest concentration prevents the growth of pathogen and the concentration before it, respectively, then examined on CRA to determine MIC and SIC (CLSI, 2013).

Table 4. Cycling conditions of the different primers during PCR of *S. aureus*.

Staphylococcus genes	Initial denaturation	Actual cycles	Final extension
16s rRNA	94°C 5 minutes	35 cycles of: Denaturation: 94°C/30 seconds Annealing: 55/45 secondsExtension: 72/30 seconds	72°C 7 minutes
Sea, *Seb* and *See*	94°C 5 minutes	35 cycles of: Denaturation: 94°C/30 seconds Annealing: 50/30 secondsExtension: 72/30 seconds	72°C 7 minutes
Sec	94°C 10 minutes	35 cycles of: Denaturation: 94°C/45 secondsAnnealing: 50/45 secondsExtension: 72/45 seconds	72°C 10 minutes
Sed	94°C 5 minutes	35 cycles of: Denaturation: 94°C/30 seconds Annealing: 48/30 secondsExtension: 72/30 seconds	72°C 7 minutes

***Pseudomonas aeruginosa pslA* expression by quantitative real-time (qRT)-PCR**

The next step was gene expression by qRT-PCR where the housekeeping gene used was 16S rRNA to find out the different levels of expression. The reaction was described by El-Demerdash and Bakry (2020). The gene expression variation was estimated by comparing the Ct of the examined sample and positive control based on "ΔΔCt" method (Yuan *et al*., 2006).

Statistical analysis

R studio was operated for both heatmap and box plot. Moreover, one-way analysis of variance SPSS version 22 for Windows was used for fold change analysis of *M. oleifera* aqueous extract against *P. aeruginosa* isolate.

Results

Bacteriological phenotypic examination

Figure 1 expresses phenotypic bacteriological results, where *P. aeruginosa* was isolated from whole samples with 30 (30%). The highest isolation rate was seen in raw milk as 48%, followed by 36% in ice cream, lower results were 20% and 16% in Kariesh cheese and processed cheese, respectively. 17% of isolates were identified biochemically as 20%, for both raw milk and Kariesh cheese, and 12% and 16% in processed cheese and ice cream, respectively. About 30% of *P. aeruginosa* isolates were biofilm producers, where 12% were intermediate, 18% of isolates were weak, while (12/17) of isolates were negative biofilm producers with 70%. *Staphylococcus aureus* was isolated from 32 (32%) of the examined samples. The highest isolation rate was detected in 44% of raw milk samples, followed by 40% of ice cream samples, lower results were recorded as 28% in Kariesh cheese samples and 16% of processed cheese samples. 24% of isolates were identified biochemically as 36% in raw milk samples, 28% in ice cream isolates, and 16% in both Kariesh cheese and processed cheese. Five of 24 (21%) *S. aureus* isolates were biofilm producers as 8% were strong producers and 12% were intermediate, while 79% were negative.

Molecular examination of suspected isolates

Figure 2 shows the results of molecular examination of suspected isolates, where all of the recovered *P. aeruginosa* isolates were positive for both *16S rRNA* gene (a confirmatory target for *P. aeruginosa*) and *nan1* gene, while 66.6%, 100%, and 100% of examined strains from raw milk, processed cheese, and ice cream samples, respectively, contained *exoS* gene, while both *toxA* and *pslA* genes were detected in isolates which were taken from raw milk and soft processed cheese samples with 33.3% and 100%, respectively (Fig. 3). All recovered *S. aureus* isolates were positive for *16S rRNA* gene (a confirmatory target for *S. aureus*), *S. aureus* isolates were examined for *SEs A* to *E*, *SEA* was positive in 100% of examined strains from all raw milk and ice cream samples, while *SEB* was detected in 50% and 100% of examined strains from raw milk and Kariesh cheese samples, respectively (Fig. 4), none of *SEC*, *SED*, and *SEE* was detected in any *S. aureus* strain.

Antibacterial activity of aqueous extract of M. oleifera

There was no MIC detected on *P. aeruginosa* and *S. aureus*. Although a biofilm of *S. aureus* did not affect by *M. oleifera* aqueous extract but *P. aeruginosa* biofilm formation MIC was 0.312 mg/ml, SIC was 0.624 mg/ml (Table 5), while relative expression of *pslA* biofilm gene treatment with MIC (3.2%) of *M. oleifera* aqueous extract revealed significant decrease in the expression level (0.40332 fold change) as shown in Table 6 and Figures 5 and 6.

Discussion

Foodborne diseases mostly affect developing countries due to bad food handling and poor sanitary (WHO, 2015). In our study, *P. aeruginosa* was isolated from 30 (30%) of the examined samples (Fig. 1). *Pseudomonas* was examined in a number of studies in Egypt, and raw milk and dairy products were reported to be contaminated with *P. aeruginosa*, with the prevalence of 48% raw milk samples, 18% soft cheese and 8% of Kareish cheese (Ibrahim *et al*., 2022), *P. aeruginosa*

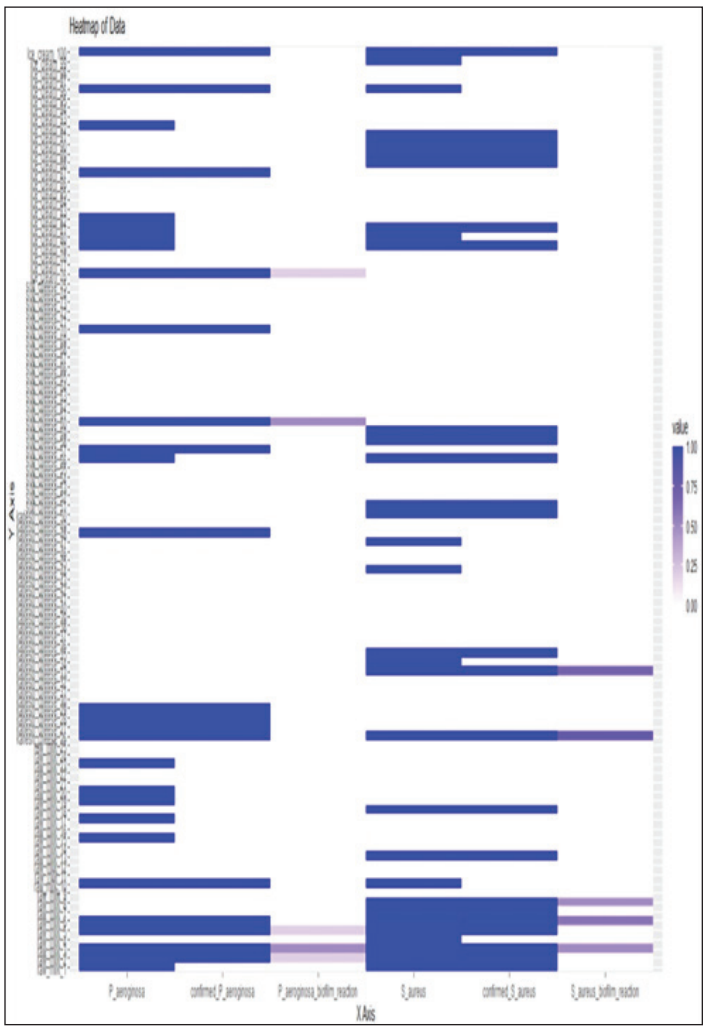

Fig. 1. Heat map that shows the overall phenotypic examination: incidence of examined food poisoning pathogens *P. aeruginosa* and *S. aureus* and their biofilm formation profile. The *X* axis of the heat map resamples examined pathogens each on its selective media, their biochemical identification, and their ability to produce biofilm, while the *Y* axis resamples type of samples. The blue color of the map indicates positive results, while the white color indicates negative results. The numbers of examined samples are found horizontally on the map.

was present in 20%,16%, and 8%, respectively, in examined raw milk, Kareish cheese, and ice cream samples (Atia *et al*., 2022), lower results were recorded by Abdel-Hameed and Saleem (2019) who found *P. aeruginosa* in 11.6% of raw milk samples and 10% of ice cream samples. About 70% of psychrotrophic pathogens like *Pseudomonas* species can be easily recovered from chilled dairy products such as raw milk and fresh cheese (Langsrud *et al*., 2003), as these are the main reservoirs of *Pseudomonas* spp. (Quintieri *et al*., 2019). *Pseudomonas* species are able to form an extracellular matrix called biofilm (Brooks and Flint, 2008), thus making them highly resistant to detergents and cleaning operations (Giaouris *et al*., 2014). About 30% of the isolates that have been recovered from examined samples in this study were biofilm producers. Higher results were obtained by Radovanic *et al*. (2020) who found that 54 (90%) of *Pseudomonas* isolates of milk samples are biofilm producers, the most prominent group was moderately biofilm producers with (70%) 42 isolates, followed by 10 (17%) weak biofilm producers, then 2 isolates (3%) were strong biofilm producers, while 6 (10%) isolates did not show any biofilm production. Different results were obtained by El-Demerdash and Bakry (2020) who used CRA to classify *P. aeruginosa* isolates taken

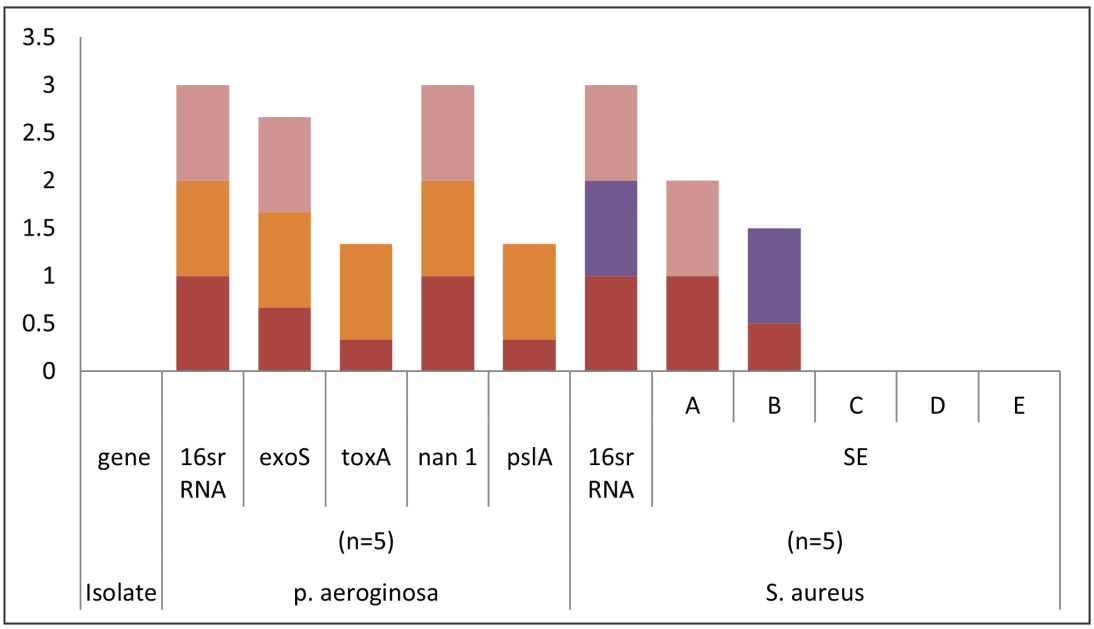

Fig. 2. Distribution of different genes in food poisoning isolates recovered from examined samples (red for raw milk, orange for processed cheese, purple for Kariesh cheese, and pink for ice cream).

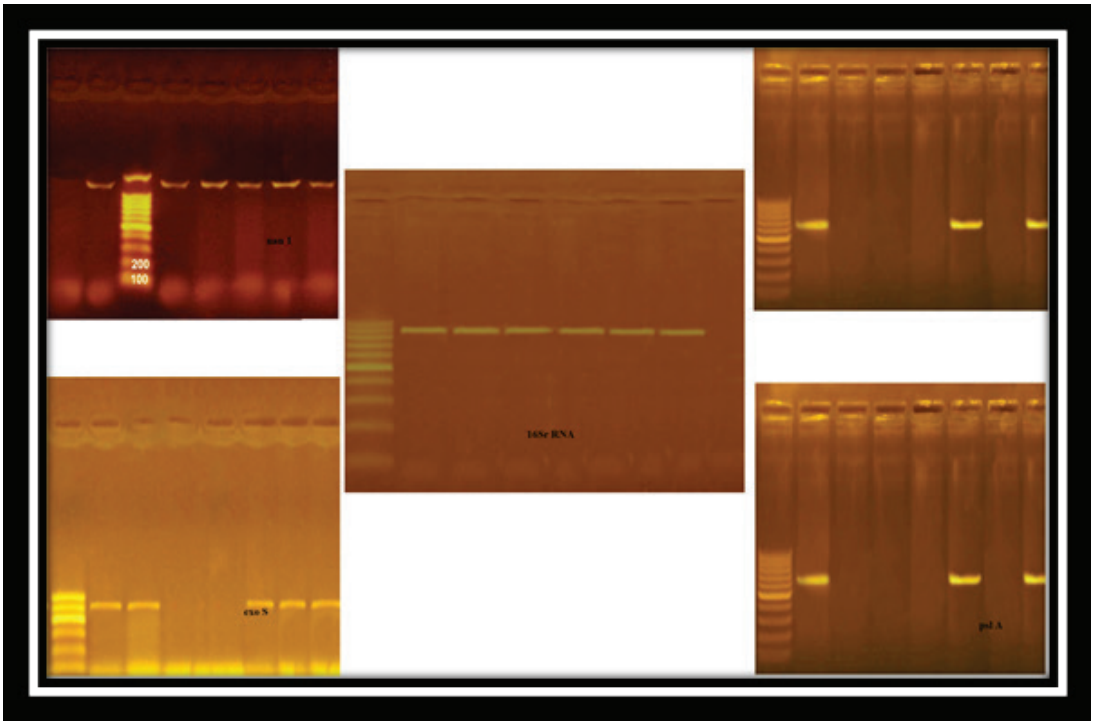

Fig. 3. *Pseudomonas aeruginosa* genes detected by conventional PCR.

from food samples and found that 21 isolates (66.6%) were biofilm producers as 19% were strong and 28.6% were intermediate, while 19% was negative. Arslan *et al*. (2011) also used CRA for slime production in *Pseudomonas* isolates taken from homemade cheese and all of them were negative. Biofilm plays a great role in *P. aeruginosa* pathogenicity causing a chronic form of infection and also resisting antimicrobial agents (Olsen, 2015). *Staphylococcus aureus* was isolated as 32% (Fig. 1) of total samples. Lower results

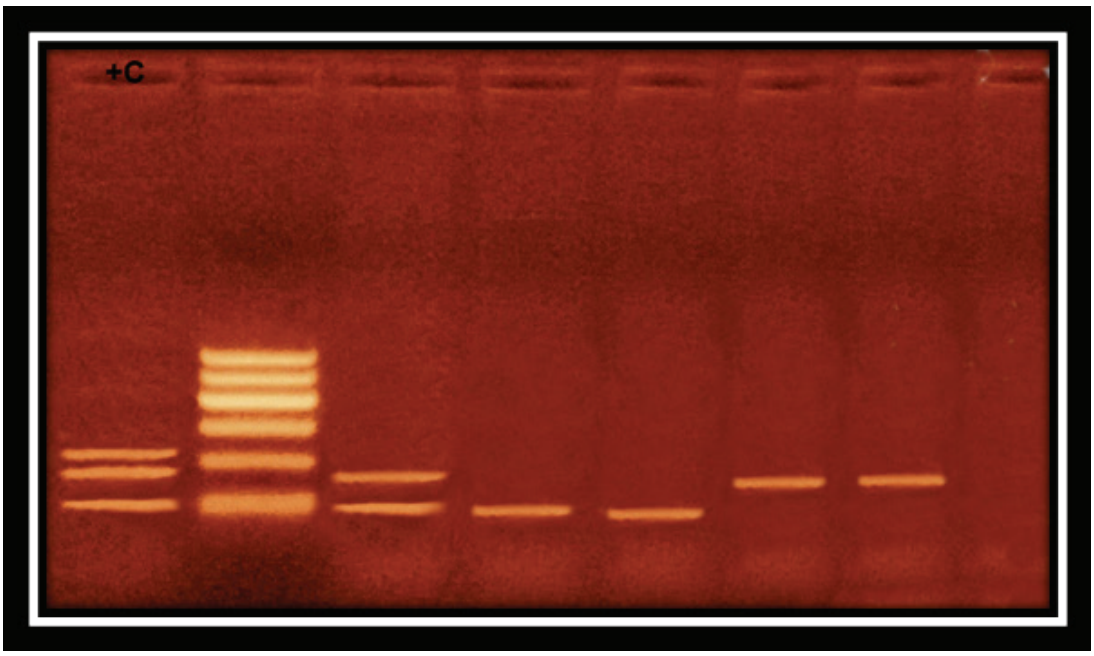

Fig. 4. *Staphylococcus aureus* enterotoxin genes detected by conventional PCR.

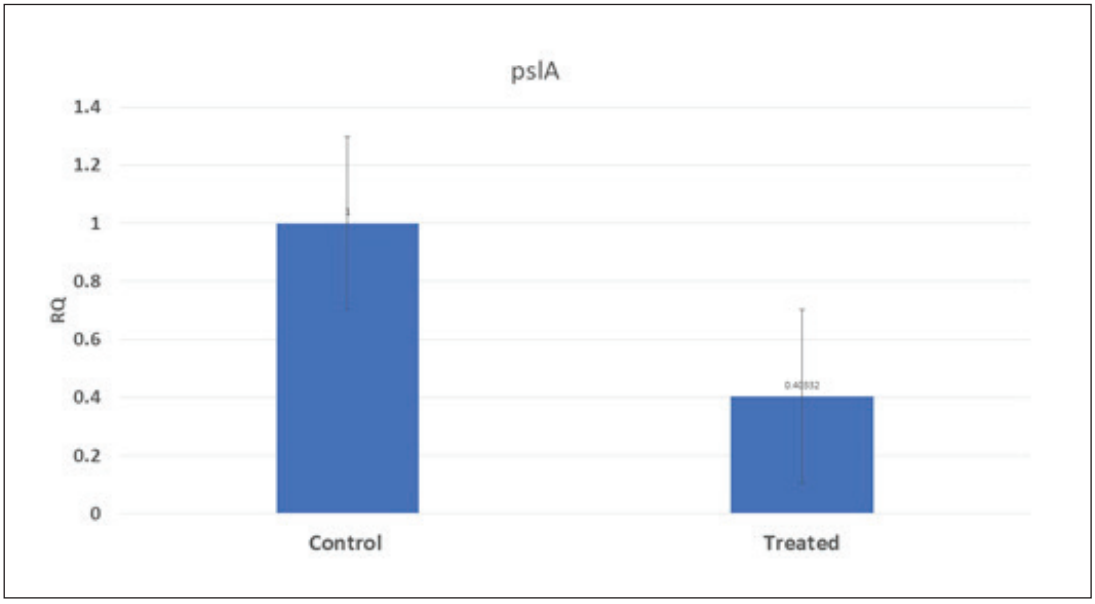

Fig. 5. Showing biofilm gene expression of *P. aeruginosa* using real time PCR after treatment with *M. oleifera*.

were obtained by Mirzaei *et al*. (2012) who found that the incidence of *S. aureus* in raw milk samples was 14% and 6% in ice cream samples, also lower results were reported in cheese samples by Baumgartner *et al*. (2014) that was (13%) and Hadi and Hassan (2013) who isolated *S. aureus* from raw milk samples with percentage (13.4%) and from white raw soft cheese samples with (20%) percentage. Higher results of *S. aureus* prevalence were 75%, 40%, and 50% in raw milk, Kareish cheese, and ice cream samples, respectively, which were obtained by Al-Ashmawy *et al*. (2016), also higher results of *S. aureus* were found by Fadel and Ismail (2015); the results were 63.3%, 40%, and 36.7% of Kareish cheese, raw buffaloes' milk, and ice cream samples, respectively. The acidity of Kareish cheese may play a great role in decreasing the incidence and prevalence rates of *S. aureus* to lower rates than those in raw milk (Al-Ashmawy *et al*., 2016). Improper personal hygiene and bad hygienic practices during dairy product manufacturing are the main causes

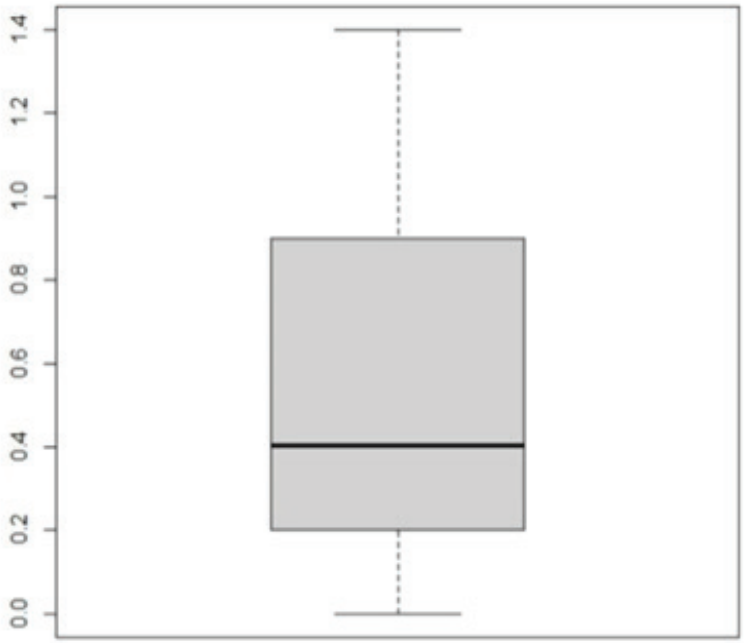

Fig. 6. Boxplot showing variation of biofilm gene expression of *P. aeruginosa* detected by using real time PCR after treatment with *M. oleifera* aquas extract.

of milk and dairy *S. aureus* contamination (Chye *et al*., 2004). About 21% of the *S. aureus* isolates in our study were biofilm producers. Different results were recorded by Avila-Novoa *et al*. (2018) who isolated *S. aureus* from dairy industry surfaces and examined these isolates on CRA, the results were that 75% of isolates were biofilm producers, 16.6% were negative and 8.3% were non characteristic phenotype. The critical point of slime formation is that the effect of antibiotics is lowered by decreasing their dose when the biofilm prevents some from passing inside the pathogen (Singh *et al*., 2010).

All recovered *P. aeruginosa* isolates were positive for *16S rRNA* gene (a confirmatory specific target for *P. aeruginosa* genus) (Ghadaksaz *et al*., 2015), while 66.6%, 100%, and 100% of examined strains from raw milk, soft processed cheese, and ice cream samples, respectively, contained *exoS* gene, that was similar to Mohammadzadeh *et al*. (2017) who reported 100% *P. aeruginosa* isolates had gene *exoA*, nearly similar results were confirmed by Ameen *et al.* (2019) where 69.7% out of *P. aeruginosa* isolates had *exoS* genes. Other studies reported lower results as Younis *et al*. (2015) found out (17.14%) of isolates harbored *exoS* gene and (36.8%) of isolates carried *exoS* gene according to the study of Banerjee *et al*. (2017). In our study, both *toxA* and *pslA* genes were only detected in isolates that were taken from raw milk samples with 33.3% and 100% of soft processed cheese, while AL-Sheikhly *et al*. (2020) recorded higher results as all examined isolates harbored *pslA* gene. For the *nan1* gene, it was detected in 100% of examined strains, lower results were detected by Tawfiq (2018) where only (41.2%) of isolates carried *nan1* gene. Many reasons such as strain virulence, immunity, and contamination level explain the different results (Khan and Cerniglia, 1994). In our study, all recovered *S. aureus* isolates were positive for *16S rRNA* gene (a confirmatory specific target for *S. aureus* genus) (Monday and Bohach, 1999), *S. aureus* isolates were examined for *SEs A* to *E*, *SEA* was positive in 100% of examined strains isolated from all raw milk and ice cream samples, while *SEB* was detected in 50% and 100% of examined strains from raw milk and Kariesh cheese samples, respectively. None of *SEC*, *SED*, and *SEE* was detected in any *S. aureus* strain. Although Al-Ashmawy *et al*. (2016) recorded the same results for *SEa*, higher rates for *SEb* and *SEc* were recorded by them as 100%. On the other hand, Oliveira *et al*. (2011) found a low percentage of the *SEc* gene in 6% of *S. aureus* isolates recovered from subclinical mastitic milk. Both Jørgensen *et al*. (2005) and Morandi *et al*. (2007) found no *SEe* in isolates recovered from milk samples which agrees with our study. These *SEs* pose a great hazard to health (WHO, 2007) as the low amount of these toxins could easily be a reason for food poisoning (Le Loir *et al*., 2003) moreover, the high heat stability of staphylococcal enterotoxin makes the incidence of food poisoning more risky (Mossong *et al*., 2015).

In our study, *M. oleifera* aqueous extract could not act as the bactericidal for *P. aeruginosa* in the MIC test; this was similar to Abalaka *et al*. (2012) who explained that point due to the high resistance nature

Table 5. Showing MIC and SIC of 0.5% *M. oleifera* aqueous extract against *S. aureus* and *P. aeruginosa.*

Isolate	SIC	MIC
P. aeruginosa	0.624 mg/ml	0.312 mg/ml
S. aureus	Not detected	Not detected

Table 6. Showing biofilm gene expression of *P. aeruginosa* using real time PCR after treatment with *M. oleifera* (*16S rRNA as a* housekeeping gene).

Group	16S rRNA	pslA	
	CT	CT	Fold change
Control +ve	24.73	27.11	1
1	24.95	28.64	0.403320

of this pathogen. On the *other* hand, 0.312 mg/ml of the aqueous leaves extract was detected as MIC on *P. aeruginosa* biofilm formation and SIC was 0.624 mg/ml, pslA biofilm gene expression of *P. aeruginosa* isolate was detected by qRT-PCR before and after treatment with the detected MIC (0.312 mg/ml) *M. oleifera* aqueous extract and we found out a revealed significant decrease in the expression level (Tables 5 and 6, Figs. 5 and 6) (0.40332-fold change). Phytochemicals inside *M. oleifera* leaves act as antibacterial materials (Bukar *et al*., 2010). Many studies examined pslA gene expression, where El-Demerdash and Bakry (2020) examined the effect of amikacin and cefotaxime on the pslA gene, the fold changes were between 0.1 and 0.7, and AL-Sheikhly *et al*. (2020) examined gentamicin at 512 µg/ml on pslA gene, but its effect was lower than expected according to them. *Staphylococcus aureus* isolate in our study was highly virulent, it was not affected by *M. oleifera* aqueous extract, which may be due to active ingredients in inadequate amounts (De Zoysa *et al*., 2019), as some of them were lost during evaporation at the step of preparation of aqueous extract (Ibrahim and Kebede, 2020).

Conclusion

Treatment with an aqueous extract of *M. oleifera* 0.5% showed a significant effect on *P. aeruginosa pslA* gene, although there was no change in the MIC test, this medicinal plant has a great antibacterial effect and needs further examinations.

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Our study had no specific grant.

Author contributions

All of the authors contributed to this study and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

None.

Data Availability

All data supporting the findings of this study are available within the manuscript.

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